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Short Communication

Let ants find them: Using ants as eDNA samplers for detecting the invasive spotted lanternfly

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Abstract

Environmental DNA (eDNA) has emerged as a valuable tool for detecting invasive species, yet its application in terrestrial ecosystems remains challenging due to uneven eDNA distribution. Ants, which forage and consume carbohydrate-rich honeydew from sap-feeding insects, may serve as effective "biological samplers" for invasive species detection. In this study, we evaluated whether ants could facilitate eDNA-based detection of the invasive spotted lanternfly (*Lycorma delicatula*, SLF), given this invasive species is well known for excreting honeydew containing detectable DNA. Worker ants were collected from SLF-infested and non-infested sites and analysed using endpoint PCR and quantitative PCR (qPCR, TaqMan assay) to detect SLF DNA. Both assays successfully detected SLF DNA in 60–100% of ant samples from infested sites, while no SLF DNA was found in ants from non-infested locations. Compared to non-ant insects, ants exhibited higher SLF DNA concentrations, suggesting that honeydew ingestion serves as the primary eDNA source. These findings demonstrate that ants can function as efficient SLF eDNA samplers, providing a scalable and cost-effective alternative to existing SLF detection methods.

Key words: Early detection, eDNA sampler, environmental DNA, foraging behaviour, honeydew, spotted lanternfly

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Introduction

Detecting invasive species using environmental DNA (eDNA) has been shown to enhance detection probability, a crucial factor for improving management efficiency and increasing the likelihood of successful eradication (Mehta et al. 2007; Keller et al. 2022). Unlike aquatic systems, eDNA in terrestrial environments is distributed unevenly. Therefore, identifying efficient methods to target areas where eDNA aggregates is essential for maximising detection success (Eichmiller et al. 2014). A notable example is the detection of the invasive spotted lanternfly (*Lycorma delicatula*, SLF), a highly destructive invasive species in the United States that poses significant agricultural threats (Urban 2020). Leveraging the presence of SLF DNA in its honeydew, Valentin et al. (2020) developed two eDNA collection techniques including spray aggregation (rinsing eDNA from shrubs and understorey vegetation) and tree rolling (removing eDNA from tree trunks and branches using a paint roller). These methods were able to collect SLF DNA from above-ground surfaces and outperformed some traditional SLF detection methods.

Ants are well known for foraging carbohydrate-rich resources, including honeydew from sap-sucking insects (Nelson and Mooney 2022). Their ecological dominance (Parr 2008) and extensive foraging ranges (Adler and Gordon 2003) suggest ants could act as SLF honeydew samplers and even potential amplifiers. Ants can retain liquid food in their gut for extended periods for later sharing with nestmates (Greenwald et al. 2018), further increasing their potential as eDNA reservoirs. If ants can serve as effective SLF eDNA samplers (Fig. 1), this "ant approach" could offer significant advantages over existing SLF eDNA methods by reducing time, device and labour requirements. For example, unlike the two previous eDNA methods requiring specialised equipment and preservation steps, ant specimens can be collected easily and processed directly. This study thus aimed to evaluate whether ants could serve as reliable SLF eDNA samplers. To test this, we analysed ant specimens collected from areas with or without SLF infestations for the presence of SLF DNA using both endpoint PCR and quantitative PCR (qPCR).

Materials and methods

Sample collection and preparation

Samples were collected from a total of six locations in Virginia, including four SLF-infested sites (Lynchburg 1, Lynchburg 2, Harrisonburg and Salem) and two sites with no recorded SLF presence (Blacksburg and South Hill). At each site, five worker ant samples were collected around an infested tree: one sample directly from the tree (e.g. ants foraging on a tree branch) and four additional samples, each from a different direction, at a distance of 5 m from the tree. Aspirators were used to collect all visible ants within a 5-minute period. To compare SLF DNA detection patterns across insect groups, non-ant insects with varying feeding habits were also collected. This comparison allowed us to assess how feeding behaviour influences SLF DNA detection and to determine whether SLF DNA detected in ants resulted from surface contamination (e.g. honeydew adhering to the surface of ants or other insects in an SLF-infested area). The inclusion of non-ant insect samples also helped validate the specificity of the molecular assays, as honeydew from other sap-sucking insects might have been present in the collected ant samples. All insect samples were preserved in absolute ethanol immediately after collection. DNA was extracted from pooled ant specimens (at least three individuals were pooled for extraction) and non-ant insect species (either single insect or pooled specimens consisting of 2–3 individuals) using the E.Z.N.A. Tissue DNA Kit, following the manufacturer's instructions. To eliminate potential external DNA contamination, all specimens underwent surface decontamination using the bleaching method described by Huszarik et al. (2023).

Molecular assays

Extracted DNA was used as a template for endpoint PCR. We followed the PCR protocol outlined in Kim et al. (2013), targeting the NADH dehydrogenase subunit 6 (ND6) gene of SLF. To ensure amplification specificity, the annealing temperature was adjusted to 56 °C. PCR products were analysed via gel electrophoresis and subsequently verified through Sanger sequencing. The resulting sequences (393 bp) were analysed using BLAST, showing > 99% similarity to published SLF sequences in GenBank, thereby validating the specificity of the primers.

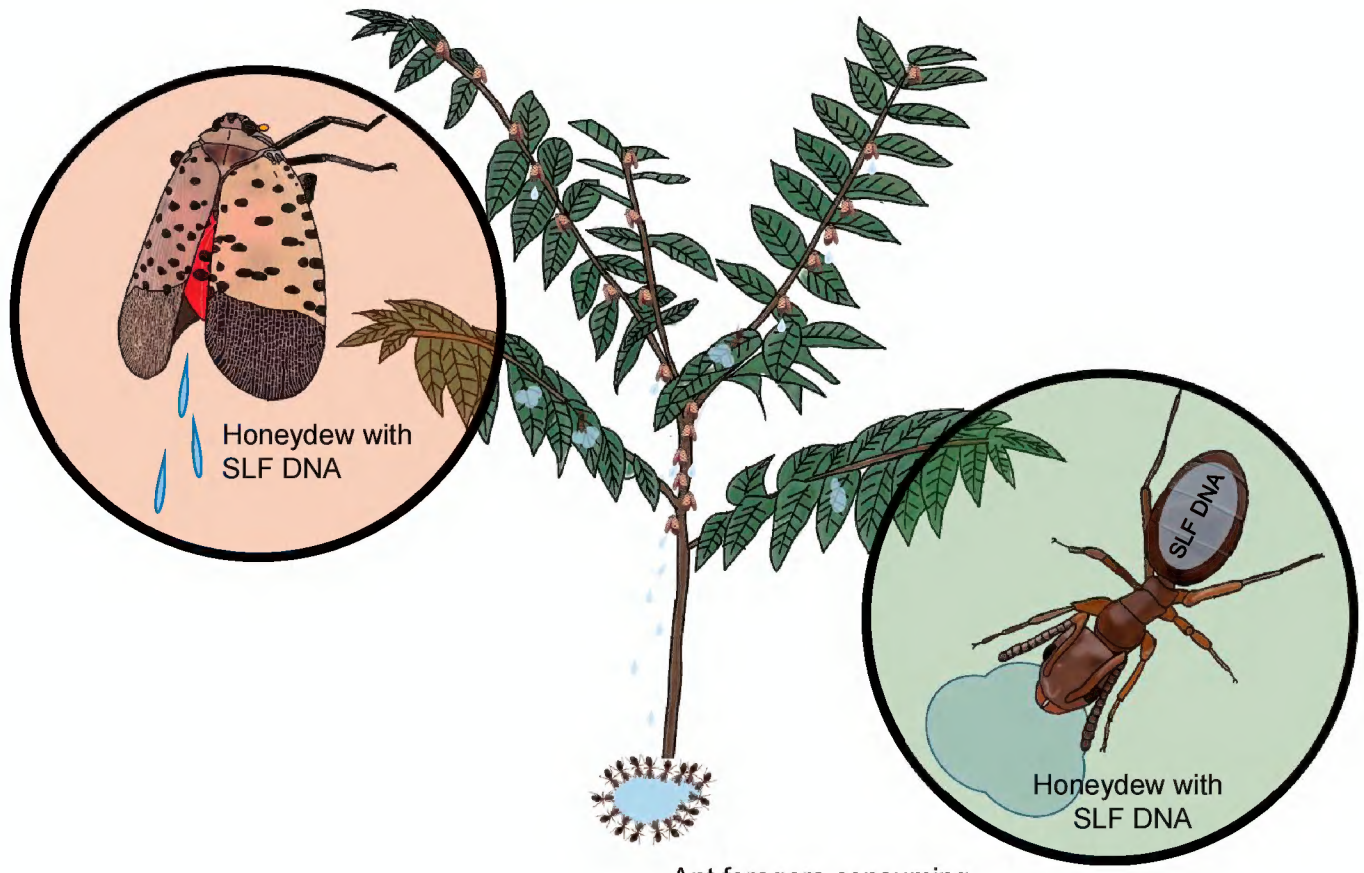

Figure 1. Ant foragers actively feed on and retain honeydew produced by spotted lanternflies (SLF), which may serve as an eDNA source for detection.

For quantitative detection, we employed a TaqMan assay following the protocols and cycling conditions described in Valentin et al. (2020). SLF DNA was analysed in technical triplicates for each sample, alongside a control sample (DNA extracted from an SLF foreleg). The control DNA was quantified using a Qubit™ Flex Fluorometer (Invitrogen, Inc.) and used to prepare a seven-point, 10-fold serial dilution (starting at 14.5 ng/µl) for standard curve generation. SLF DNA concentrations per reaction were estimated, based on the standard curves (efficiencies = 80.35–87.36%; R^2 = 0.9948–0.9952). To ensure the reliability of results, each qPCR run included no-template controls (NTCs) using molecular-grade water as a negative control. One NTC exhibited a Ct value slightly above 43. Samples were classified as positive if they produced a visible band in endpoint PCR or a detectable Ct value (< 41) in qPCR across all triplicates.

Results

Both endpoint PCR and qPCR successfully detected SLF DNA in nearly all the ant samples. Worker ant samples collected from the infested areas showed detection rates ranging from 60–100% using PCR and 80–100% with qPCR (Fig. 2). None of the ant samples collected at the sites with no SLF was positive for SLF DNA using either method (Fig. 2). No SLF DNA was detected in non-ant insect samples (including leafhoppers, lady beetles, stinkbugs and whiteflies) using PCR, consistent with the primer's specificity to SLF. Compered to PCR, qPCR appears to produce higher detection rates in three non-ant insects: leafhoppers (66.6%, 2/3), ladybeetles (50%, 3/6) and stinkbugs (100%, 3/3). However, SLF DNA concentrations detected in ants were higher than in all non-ant insect samples (Fig. 3). While these results may be indicative of the presence of SLF DNA in the non-ant insects, it is important to note that some of these signals may have resulted from short molecular artefacts such as primer dimers.

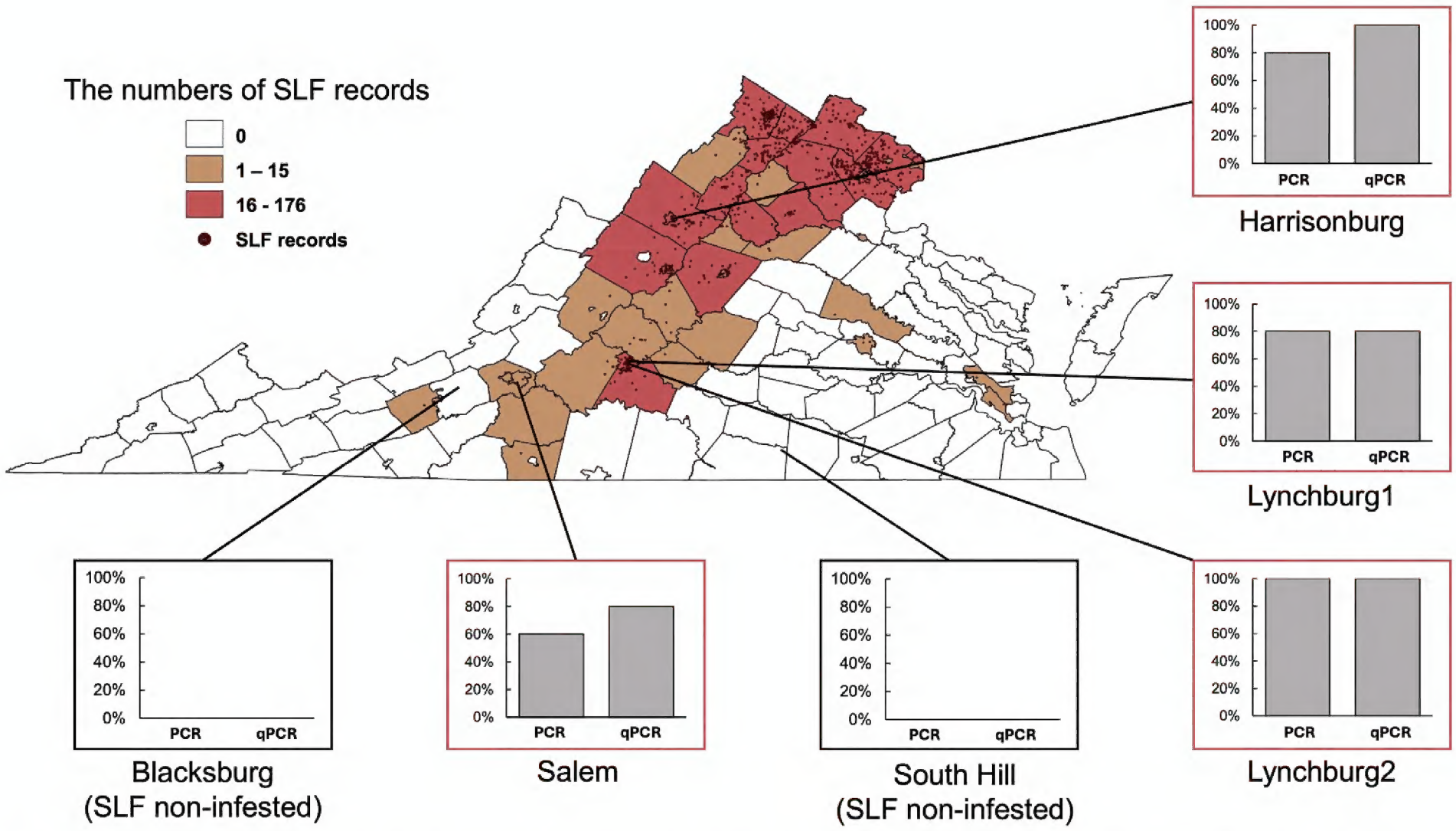

Figure 2. SLF DNA detection rates in ant samples collected from each site, determined by PCR and qPCR (TaqMan assay). A choropleth map of spotted lanternfly (SLF) distribution across Virginia is overlaid with a point density map showing SLF infestation sites. The choropleth layer was generated using QGIS 3.26.3 with kernel density estimation (KDE) and a kernel distance of 20 km. Occurrence data consist of 1,487 human observation records from July 2019 to August 2024 in Virginia, United States (Occurrence download: https://doi.org/10.15468/dl.vurasf, accessed via GBIF.org on 2024-09-12).

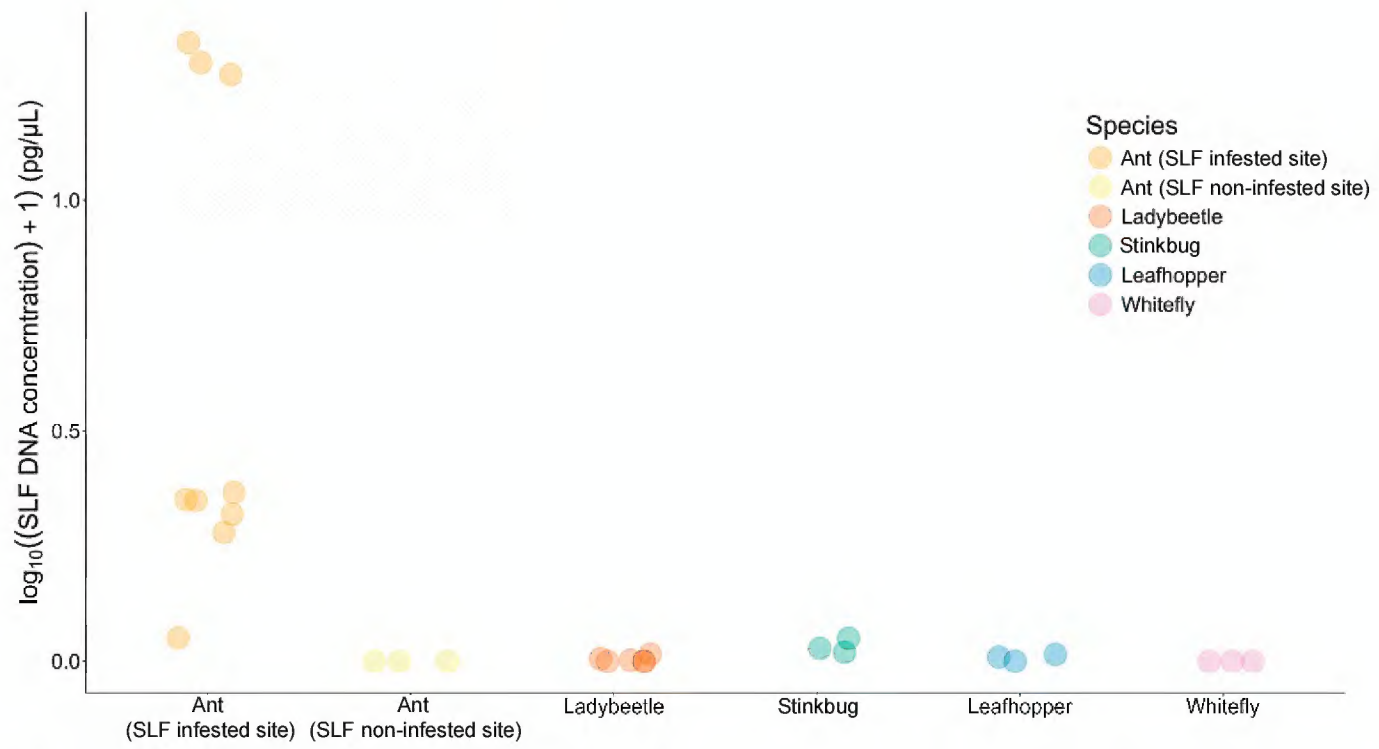

Figure 3. Detection and quantification of SLF DNA in various insect taxa. The detection rates of SLF DNA in ants (including those collected from SLF-infested and non-infested sites), ladybeetles, stinkbugs, leafhoppers and whiteflies were analysed using TaqMan assay.

Discussion

This study demonstrates that ants serve as effective SLF eDNA samplers, as evidenced by their consistently high SLF detection rates using both PCR and qPCR. Compared to non-ant insect species, which generally lack direct interactions with SLF honeydew, ants exhibited significantly higher SLF DNA

concentrations. This strongly suggests that honeydew consumption plays a key role in the successful detection of SLF DNA. The effectiveness of ants as SLF eDNA samplers is likely due to their foraging behaviour, which involves actively seeking and retaining carbohydrate-rich foods, including honeydew. This behaviour enables ants to aggregate both honeydew and the DNA contained within it. While we cannot fully rule out the possibility of some SLF DNA detections originating from scavenged SLF tissues, it is unlikely to be the primary source. Worker ants typically transport solid prey back to the nest for larvae to digest, whereas liquid food such as honeydew is retained and shared amongst workers (Greenwald et al. 2018; Fujioka et al. 2023), making the eDNA source in sampled ants more plausible. Leveraging ants' foraging behaviour is particularly advantageous for detecting low-density SLF populations. Early SLF infestations are often cryptic, requiring a method capable of effectively aggregating and concentrating eDNA from the environment. Given ants' extensive foraging ranges (Paulson and Akre 1991), they can cover large areas while searching for food, increasing the likelihood of detecting SLF even when densities are low. While foraging ranges vary across species, some of the ant species (e.g. *Camponotus* ants) collected in this study are known to forage 10–30 m from their nests (Buczkowski 2011). Additionally, their polydomous nature (i.e. colonies are spread across multiple spatially separated nests; Robinson (2014)) may further enhance the detection coverage. This suggests that using ants to detect SLF may extend the detection range beyond the core infestation. Compared to previous eDNA methods (Valentin et al. 2020), the ant approach offers several key advantages: (1) The requirement of filtration or specialised preservation is eliminated, reducing both time and cost; (2) The scalability of this method makes it an ideal candidate for large-scale SLF surveillance. While ants were collected manually using aspirators in this study, well-established ant collection techniques, such as lure stations deployed along transects, could be readily implemented in areas requiring large-scale monitoring; (3) This approach is adaptable across a broad range of environments as ants are widely distributed in diverse habitats. Future research should focus on testing the sensitivity of this method for detecting SLF at low densities and optimising lure-based ant collection techniques to support large-scale monitoring. These efforts will be essential for addressing the ongoing rapid expansion of SLF across the United States.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

W.-J. Lin and C.-C. S. Yang conceived the study; C.-C. S. Yang secured funding and oversaw the project; W.-J. Lin, F.-L. C. Liu, L. Cho and C.-C. S. Yang collected the data; W.-J. Lin and C.-C. S. Yang analysed the data and wrote the manuscript. All authors edited, reviewed and approved the final manuscript for submission.

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Data availability

All of the data that support the findings of this study are available in the main text.

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